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<b>(21) International Application Number:</b> PCT/US97/14866 <b>(22) International Filing Date:</b> 22 August 1997 (22.08.97)  <b>(30) Priority Data:</b> 60/024,349           23 August 1996 (23.08.96)       US 60/031,569           4 December 1996 (04.12.96)       US 60/036,939           6 February 1997 (06.02.97)       US  <b>(71) Applicant:</b> CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608-2916 (US).  <b>(72) Inventor:</b> RANDAZZO, Filippo; 6363 Christie Avenue #1401, Emeryville, CA 94608 (US).  <b>(74) Agents:</b> POTTER, Jane, E., R. et al.; Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608-2916 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> HUMAN POLYHOMEOTIC 1(hphl) ACTS AS A TUMOR SUPPRESSOR  <b>(57) Abstract</b>  <p>A novel human tumor suppressor gene termed polyhomeotic 1 is disclosed. The human polyhomeotic 1 gene and protein can be used as therapeutic and diagnostic tools for proliferative and developmental disorders. The gene can also be used to identify a p13 region of human chromosome 12.</p>		

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**HUMAN POLYHOMEOTIC 1 (*hph1*) ACTS AS A TUMOR SUPPRESSOR**

This application claims the benefit of the following copending provisional applications: Serial No. 60/024,349, filed August 23, 1996, Serial No. 60/031,569, filed December 4, 1996, and Serial No. 60/036,939, filed February 6, 1997, each of which is incorporated by reference herein.

**TECHNICAL AREA OF THE INVENTION**

The invention relates to the area of tumor suppression. More particularly, the invention relates to tumor suppressor genes and proteins.

**BACKGROUND OF THE INVENTION**

Mutations in tumor suppressor genes play an important role in the development of neoplasias. Manipulation of tumor suppressor gene expression can be used to prevent or treat neoplasias. Detection of mutations in tumor suppressor genes can also be used to detect neoplastic cells and genetic predispositions to neoplasias. Thus, there is a need in the art for the identification of mammalian tumor suppressor genes which can be used in methods of diagnosing, prognosing, and treating neoplastic cells in humans and other mammals.

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**SUMMARY OF THE INVENTION**

It is an object of the invention to provide an isolated and purified human polyhomeotic 1 protein.

5 It is another object of the invention to provide an isolated and purified human polyhomeotic 1 polypeptide.

It is yet another object of the invention to provide an *hph1* fusion protein.

It is still another object of the invention to provide a preparation of antibodies.

It is yet another object of the invention to provide an isolated and purified subgenomic polynucleotide.

10 It is still another object of the invention to provide an expression construct for expressing all or a portion of a human polyhomeotic 1 protein.

It is a further object of the invention to provide a homologously recombinant cell comprising a DNA construct.

15 It is even another object of the invention to provide a method of identifying neoplastic tissue of a human.

It is another object of the invention to provide a method to aid in the diagnosis or prognosis of neoplasia in a human.

~~It is yet another object of the invention to provide a method to aid in detecting~~  
a genetic predisposition to neoplasia in a human.

20 It is still another object of the invention to provide a method of identifying a human chromosome 12.

It is even another object of the invention to provide a therapeutic composition for restoring a wild-type human polyhomeotic 1 function to a cell which lacks that function.

25 It is another object of the invention to provide a method of treating proliferative disorders.

It is still another object of the invention to provide a method of inducing a cell to differentiate.

30 These and other objects of the invention are provided by one or more of the embodiments described below.

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One embodiment of the invention provides an isolated and purified human polyhomeotic 1 protein. The isolated and purified human polyhomeotic 1 has the amino acid sequence shown in SEQ ID NO:2.

5 Another embodiment of the invention provides an isolated and purified human polyhomeotic 1 polypeptide. The isolated and purified human polyhomeotic 1 polypeptide consists of at least 22 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2.

10 Yet another embodiment of the invention provides an *hph1* fusion protein. The *hph1* fusion protein comprises a first protein segment and a second protein segment fused together by means of a peptide bond. The first protein segment consists of at least 8 contiguous amino acids of a human polyhomeotic 1 protein.

Still another embodiment of the invention provides a preparation of antibodies which specifically bind to a human polyhomeotic 1 protein.

15 Even another embodiment of the invention provides an isolated and purified subgenomic polynucleotide. The isolated and purified subgenomic polynucleotide consists of at least 10 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1.

20 Yet another embodiment of the invention provides an expression construct for expressing all or a portion of a human polyhomeotic 1 protein. The expression construct comprises a promoter and a polynucleotide segment. The polynucleotide segment encodes at least 8 contiguous amino acids of a human polyhomeotic 1 protein. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter.

25 A further embodiment of the invention provides a homologously recombinant cell. The homologously recombinant cell comprises an incorporated new transcription unit wherein the new transcription unit comprises an exogenous regulatory sequence, an exogenous exon, and a splice donor site. The transcription unit is located upstream of a coding sequence of an *hph1* gene. The exogenous regulatory sequence directs transcription of the coding sequence of the *hph1* gene. The new transcription unit is  
30 incorporated into the genome using segments of homologous DNA from the *hph1*

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gene. The new transcription unit can be used to turn the *hph1* gene on or off as desired.

Still another embodiment of the invention provides a method of identifying a neoplastic tissue of a human. The method comprises comparing the expression of a polyhomeotic 1 gene in a first tissue of a human suspected of being neoplastic with the expression of a polyhomeotic 1 gene in a second tissue of the human which is normal. Underexpression of the human polyhomeotic 1 gene in the first tissue identifies the first tissue as being neoplastic.

Even another embodiment of the invention provides a method to aid in the diagnosis or prognosis of neoplasia in a human. The method comprises comparing a polyhomeotic 1 gene, mRNA, or protein in a first tissue of a human suspected of being neoplastic with a polyhomeotic 1 gene, mRNA, or protein in a second tissue of a human which is normal. A difference between the polyhomeotic 1 genes, mRNAs, or proteins in the first and second tissues indicates neoplasia in the first tissue.

Another embodiment of the invention provides a method to aid in detecting a genetic predisposition to neoplasia in a human. The method comprises comparing a polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of a human with a wild-type human polyhomeotic 1 gene, mRNA, or protein. A difference between the polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of the human and the wild-type human polyhomeotic 1 gene, mRNA, or protein indicates a genetic predisposition to neoplasia in the human.

Yet another embodiment of the invention provides method of identifying a human chromosome 12. The method comprises the steps of contacting a preparation of metaphase human chromosomes with a nucleotide probe comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1 and detecting a region of a chromosome which specifically hybridizes to the nucleotide probe. A region of a chromosome which specifically hybridizes to the nucleotide probe is identified as a region of human chromosome 12.

Even another embodiment of the invention provides a therapeutic composition for restoring a wild-type human polyhomeotic 1 function to a cell which lacks that function. The therapeutic composition comprises all or a portion of a wild-type

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human polyhomeotic 1 gene or expression product and a pharmaceutically acceptable carrier. Said all or a portion of the wild-type human polyhomeotic 1 gene or expression product is capable of restoring a wild-type polyhomeotic 1 function to a cell.

5           Still another embodiment of the invention provides a method of treating proliferative disorders. The method comprises the step of administering to a human a composition comprising all or a portion of a wild-type human polyhomeotic 1 gene or expression product. Said all or a portion of the wild-type human polyhomeotic 1 gene or expression is capable of restoring or enhancing a wild-type polyhomeotic 1  
10          function to a cell.

          A further embodiment of the invention provides a method of inducing a cell to differentiate. The method comprises the step of contacting a progenitor cell with a composition comprising all or a portion of a human polyhomeotic 1 gene or expression product. Said all or a portion of the human polyhomeotic 1 gene or expression  
15          product is capable of inducing differentiation of the cell.

          The present invention thus provides the art with a novel human tumor suppressor gene, called "polyhomeotic 1" (*hph1*). The *hph1* gene and protein can be used, *inter alia*, as therapeutic and diagnostic tools for proliferative and developmental disorders and to identify a p13 region of a human chromosome 12.

## 20       BRIEF DESCRIPTION OF THE DRAWINGS

          Figure 1. Northern blots probed with an *hph1* nucleotide probe. The figure depicts *hph1* mRNA expression in human tissues and cancer cell lines.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

25       It is a discovery of the present invention that *hph1* functions as a tumor suppressor and thus is implicated in neoplasias and other proliferative disorders, such as dysplasias and hyperplasias. The *hph1* gene, protein, and mRNA can be used as a diagnostic and therapeutic tool for these disorders. Coding sequences of *hph1* can also be used to identify human chromosome 12.

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The coding region of the *hph1* gene has the nucleotide sequence shown in SEQ ID NO:1. Other (degenerate) sequence encoding the same amino acid sequence and those nucleotide sequences which vary by up to 10% are included herein. Typically these can be confirmed by hybridization under stringent conditions. The *hph1* gene maps to human chromosome 12p13, a region frequently lost in non-small cell lung cancer and breast cancer. While not wanting to be bound by any particular theory, it is believed that there are lethal alleles of *hph1* which are involved in neoplasias, such as non-small cell lung carcinoma, breast cancer, lymphoma, melanoma, or adenocarcinoma. In addition, it is believed that *hph1* is involved in dyplastic disorders, such as cervical epithelial dysplasia, and hyperplastic disorders, such as breast, prostate, or thyroid hyperplasias.

Human polyhomeotic 1 protein has the amino acid sequence shown in SEQ ID NO:2. Any naturally occurring variants of this sequence that may occur in human tissues and which have tumor suppressive or antiproliferative activity are within the scope of this invention. Thus, reference herein to either the nucleotide or amino acid sequence of *hph1* includes reference to naturally occurring variants of these sequences. Nonnaturally occurring variants which differ by as much as four amino acids and retain biological function are also included here. Preferably the changes are conservative amino acid changes, *i.e.*, changes of similarly charged or uncharged amino acids.

Human polyhomeotic 1 mRNA comprises two major transcripts which measure 4.4 kb and 6.5 kb on Northern blots of human polyA<sup>+</sup> RNA probed with an *hph1* nucleotide probe (Figure 1). The 4.4 and 6.5 kb transcripts are expressed, *inter alia*, at highest levels in adult thymus and testis; at lower levels in the heart, prostate, ovary, small intestine, peripheral blood lymphocytes, skeletal muscle, and pancreas; and at very low levels in the lung, liver, and kidney. The 4.4 and 6.5 kb *hph1* mRNA transcripts are also expressed in human cancer cell lines, such as SW480 (colon carcinoma), G361 (melanoma), and Burkitt's lymphoma Raji.

Human polyhomeotic 1 polypeptides comprise at least 22, 25, 30, or 35 contiguous amino acids of the amino acid sequence shown in SEQ ID NO:2. Both full-length *hph1* protein and *hph1* polypeptides are useful for generating antibodies against *hph1* amino acid sequences. Human polyhomeotic 1 proteins and polypeptides



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can be isolated and purified from human cells such as thymus, testis, heart, prostate, ovary, small intestine, peripheral blood lymphocytes, skeletal muscle, pancreas, lung, liver, and kidney. Preferably, the protein or polypeptides are purified from thymus or testis.

5 Polyhomeotic 1 proteins or polypeptides can be purified by any method known in the art. These methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, crystallization, electrofocusing, and preparative gel electrophoresis. The skilled artisan can readily select methods which will result in a  
10 preparation of hph1 protein or polypeptide which is substantially free from other proteins and from carbohydrates, lipids, or subcellular organelles. A preparation of isolated and purified hph1 protein is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations may be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

15 Polyhomeotic 1 proteins and polypeptides can be produced by recombinant DNA methods or by synthetic chemical methods. For production of recombinant hph1 proteins or polypeptides, *hph1* coding sequences selected from the nucleotide sequence shown in SEQ ID NO:1 can be expressed in known prokaryotic or eukaryotic expression systems. Bacterial, yeast, insect, or mammalian expression  
20 systems can be used, as is known in the art. Synthetic chemical methods, such as solid phase peptide synthesis, can be used to synthesize hph1 protein or polypeptides.

Fusion proteins containing at least eight contiguous hph1 amino acids can also be constructed. Human polyhomeotic 1 fusion proteins are useful for generating antibodies against hph1 amino acid sequences and for use in various assay systems.  
25 For example, hph1 fusion proteins can be used to identify proteins which interact with hph1 protein and influence its function. Physical methods, such as protein affinity chromatography, or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art, and can be used *inter alia* as drug screens.

30 An hph1 fusion protein comprises two protein segments fused together by means of a peptide bond. The first protein segment comprises at least 8, 10, 12, 15,

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or 20 contiguous amino acids of an *hph1* protein. The amino acids can be selected from the amino acid sequence shown in SEQ ID NO:2 or from a naturally or nonnaturally occurring biologically active variant of that sequence. The first protein segment can also be a full-length *hph1* protein. The second protein segment can be a full-length protein or a protein fragment or polypeptide. The fusion protein can be labeled with a detectable marker, as is known in the art, such as a radioactive, fluorescent, chemiluminescent, or biotinylated marker. The second protein segment can be an enzyme which will generate a detectable product, such as  $\beta$ -galactosidase or other enzymes which are known in the art.

Techniques for making fusion proteins, either recombinantly or by covalently linking two protein segments, are also well known. Recombinant DNA methods can be used to construct *hph1* fusion proteins, for example, by making a DNA construct which comprises *hph1* coding sequences selected from SEQ ID NO:1 in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as described below.

Isolated and purified *hph1* proteins, polypeptides, or fusion proteins can be used as immunogens, to obtain a preparation of antibodies which specifically bind to an *hph1* protein. The antibodies can be used to detect wild-type *hph1* proteins in human tissue. The antibodies can also be used to detect the presence of mutations in the *hph1* gene which results in underexpression of the *hph1* protein or in *hph1* proteins with altered size or electrophoretic mobilities.

Preparations of polyclonal and monoclonal *hph1* antibodies can be made using standard methods known in the art. The antibodies specifically bind to epitopes present in *hph1* proteins having the amino acid sequence shown in SEQ ID NO:2. Preferably, the *hph1* epitopes are not present in other human proteins. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids. Antibodies which specifically bind to *hph1* proteins provide a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in Western blots or other immunochemical assays. Preferably, antibodies which specifically bind *hph1* proteins do not detect other

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proteins in immunochemical assays and can immunoprecipitate *hph1* proteins from solution.

Human polyhomeotic 1 antibodies can be purified by methods well known in the art. Preferably, the antibodies are affinity purified, by passing antiserum over a column to which an *hph1* protein, polypeptide, or fusion protein is bound. The bound antibodies can then be eluted from the column, for example using a buffer with a high salt concentration.

Purified and isolated *hph1* subgenomic polynucleotides can be used, *inter alia*, as primers to obtain additional copies of the polynucleotides, to express *hph1* mRNA, protein, polypeptides, or fusion proteins, and as probes for identifying wild-type and mutant *hph1* coding sequences. The probes can also be used to identify the short arm of a human chromosome 12, as described below.

Purified and isolated *hph1* subgenomic polynucleotides of the invention comprise at least 10 contiguous nucleotides selected from SEQ ID NO:1. Subgenomic *hph1* polynucleotides according to the invention contain less than a whole chromosome. Preferably, the polynucleotides are intron-free.

Subgenomic *hph1* polynucleotides can be isolated and purified free from other nucleotide sequences using standard nucleic acid purification techniques. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprise the *hph1* coding sequences. Isolated polynucleotides are in preparations which are free or at least 90% free of other molecules.

A number of terms used in the art of genetic engineering and protein chemistry are used herein with the following defined meanings.

Two nucleic acid fragments are "homologous" if they are capable of hybridizing to one another under hybridization conditions described in Maniatis et al., op. cit., pp. 320-323. However, by using the following wash conditions--2 x SCC, 0.1% SDS, room temperature twice, 30 minutes each; then 2 x SCC, 0.1% SDS, 50 ° C. once, 30 minutes; then 2 x SCC, room temperature twice, 10 minutes each--homologous sequences can be identified that contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches. These degrees of

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homology can be selected by using more stringent wash conditions for identification of clones from gene libraries (or other sources of genetic material), as is well known in the art.

5 A DNA fragment is "derived from" an *hph1*-encoding DNA sequence if it has the same or substantially the same basepair sequence as a region of the coding sequence of the entire *hph1* molecule. Preferably the DNA sequence has at least 95 % or preferably 98 % or 99 % identity with *hph1*. Substantially the same means, when referring to biological activities, that the activities are of the same type although they may differ in degree. When referring to amino acid sequences, substantially the same means that the molecules in question have similar biological properties and  
10 preferably have at least 85 %, 90 %, or 95 % homology in amino acid sequences. More preferably, the amino acid sequences are at least 98 % identical. In other uses, substantially the same has its ordinary English language meaning. A protein is "derived from" an *hph1* molecule if it has the same or substantially the same amino acid  
15 sequence as a region of the *hph1* molecule.

Protein *hph1* both glycosylated and unglycosylated, or polypeptide derivatives thereof, may be used for producing antibodies, either monoclonal or polyclonal, specific to *hph1*. By polypeptide derivatives is meant polypeptides differing in length  
from natural *hph1* and containing five or more amino acids from *hph1* in the same  
20 primary order as found in *hph1* as obtained from a natural source. Polypeptide molecules having substantially the same amino acid sequence as *Hph1* but possessing minor amino acid substitutions that do not substantially affect the ability of the *Hph1* polypeptide derivatives to interact with *Hph1*-specific molecules, such as antibodies, are within the definition of *Hph1*. Derivatives include glycosylated forms, aggregative  
25 conjugates with other molecules and covalent conjugates with unrelated chemical moieties. Derivatives also include allelic variants, species variants, and muteins. Truncations or deletions of regions which do not affect biological function are also encompassed. Covalent derivatives are prepared by linkage of functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue by means  
30 known in the art.

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Hph1-specific molecules include polypeptides such as antibodies that are specific for the Hph1 polypeptide containing the naturally occurring Hph1 amino acid sequence. By "specific binding polypeptide" is intended polypeptides that bind with Hph1 and its derivatives and which have a measurably higher binding affinity for the target polypeptide, *i.e.*, Hph1 and polypeptide derivatives of Hph1, than for other polypeptides tested for binding. Higher affinity by a factor of 10 is preferred, more preferably a factor of 100. Binding affinity for antibodies refers to a single binding event (*i.e.*, monovalent binding of an antibody molecule). Specific binding by antibodies also means that binding takes place at the normal binding site of the molecule's antibody (at the end of the arms in the variable region).

As discussed above, minor amino acid variations from the natural amino acid sequence of Hph1 are contemplated as being encompassed by the term Hph1; in particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into four families: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding properties of the resulting molecule, especially if the replacement does not involve an amino acid at a binding site involved in an interaction of Hph1. Whether an amino acid change results in a functional peptide can readily be determined by assaying the properties of the Hph1 polypeptide derivative.

Complementary DNA encoding hph1 proteins can be made using reverse transcriptase, with hph1 mRNA as a template. The polymerase chain reaction (PCR) can be used to obtain the polynucleotides, using either human genomic DNA or cDNA as a template. Alternatively, synthetic chemistry techniques can be used to synthesize

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the polynucleotide molecules of the invention. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a polyhomeotic 1 protein having the amino acid sequence shown in SEQ ID NO:2. All such nucleotide sequences are within the scope of the present invention.

5           An *hph1* subgenomic polynucleotide of the present invention can be used in an expression construct, to express all or a portion of an *hph1* protein in a host cell. The host cell comprising the expression construct can be prokaryotic or eukaryotic. A variety of host cells for use in bacterial, yeast, insect, and human expression systems are available and can be used to express the expression construct. The expression  
10 constructs can be introduced into the host cells using any technique known in the art. These techniques include transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, and calcium phosphate-mediated transfection.

15           The expression construct comprises a promoter which is functional in the particular host cell selected. The skilled artisan can readily select an appropriate promoter from the large number of cell type-specific promoters known and used in the art. The expression construct can also contain a transcription terminator which is functional in the host cell. The expression construct comprises a polynucleotide  
20 segment which encodes all or a portion of an *hph1* protein. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter. The expression construct can be linear or circular and can contain sequences, if desired, for autonomous replication.

25           The *hph1* gene maps to human chromosome region 12p13. Thus, the subgenomic polynucleotides of the invention can be used to identify this chromosome region in metaphase spreads of human chromosomes. Preparations of human metaphase chromosomes can be prepared using standard cytogenetic techniques from human primary tissues or cell lines. Nucleotide probes comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1  
30 are used to identify the human chromosome. The nucleotide probes can be labeled, for example, with a radioactive, fluorescent, biotinylated, or chemiluminescent label, and

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detected by well known methods appropriate for the particular label selected. Protocols for hybridizing nucleotide probes to preparations of metaphase chromosomes are well known in the art. A nucleotide probe will hybridize specifically to nucleotide sequences in the chromosome preparations which are complementary to the nucleotide sequence of the probe. A probe which hybridizes specifically to human chromosome region 12p13 hybridizes to nucleotide sequences present in the *hphI* gene and not to nucleotide sequences present in other human genes. A probe which hybridizes specifically to an *hphI* gene provides a detection signal at least 5-, 10-, or 20-fold higher than the background hybridization provided with non-*hphI* coding sequences.

A human chromosome which specifically hybridizes to an *hphI* nucleotide probe is identified as a human chromosome 12. Preferably, the nucleotide probe identifies the short arm of human chromosome 12. More preferably, the nucleotide probe identifies a p13 region of human chromosome 12.

The present invention also provides a method to identify neoplastic tissue in a human. The expression of an *hphI* gene can be compared between a first tissue which is suspected of being neoplastic and a second tissue of the human which is normal. The normal tissue can be any tissue of the human, especially those which express the *hphI* gene including, but not limited to, thymus, testis, heart, prostate, ovary, small intestine, peripheral blood lymphocytes, skeletal muscle, pancreas, lung, liver, and kidney. The tissue suspected of being neoplastic can be derived from a different tissue type of the human, but preferably it is derived from the same tissue type, for example an intestinal polyp or other abnormal growth. A difference between the *hphI* gene, mRNA, or protein in the two tissues which are compared, for example in molecular weight, amino acid or nucleotide sequence, or relative abundance, indicates a somatic mutation in the *hphI* gene in the tissue of the human which was suspected of being neoplastic.

The *hphI* genes in the two tissues can be compared by any means known in the art. For example, the two genes can be sequenced, and the sequence of the *hphI* gene in the tissue suspected of being neoplastic can be compared with the wild-type sequence in the normal tissue. The *hphI* genes or portions of the *hphI* genes in the two tissues can be amplified, for example using nucleotide primers selected from the

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nucleotide sequence shown in SEQ ID NO:1 in the polymerase chain reaction (PCR). The amplified genes or portions of genes can be hybridized to nucleotide probes selected from the nucleotide sequence shown in SEQ ID NO:1. The nucleotide probes can be labeled by a variety of methods, such as radiolabeling, biotinylation, or labeling with fluorescent or chemiluminescent tags, and detected by standard methods known in the art.

Alternatively, *hph1* mRNA in the two tissues can be compared. PolyA+ RNA can be isolated from the two tissues as is known in the art. For example, one of skill in the art can readily determine differences in the size or amount of *hph1* mRNA transcripts between the two tissues that are compared, using Northern blots and nucleotide probes selected from the nucleotide sequence shown in SEQ ID NO:1. Underexpression of *hph1* mRNA in a tissue sample suspected of being neoplastic compared with the expression of *hph1* mRNA in a normal tissue is indicative of neoplasia.

Any method for analyzing proteins can be used to compare two *hph1* proteins from matched samples. The sizes of the *hph1* proteins in the two tissues can be compared, for example, using the antibodies of the present invention to detect *hph1* proteins in Western blots of protein extracts from the two tissues. Other changes, such as expression levels and subcellular localization, can also be detected immunologically. A lower *hph1* protein expression level in a tissue suspected of being neoplastic compared with the *hph1* protein expression level in a normal tissue is indicative of neoplasia.

Similarly, comparison of *hph1* gene sequences or of *hph1* gene expression products, *e.g.*, mRNA and protein, between a tissue of a human which is suspected of being neoplastic and a normal tissue of a human can be used to diagnose or prognose neoplasia in the human. Such comparisons of *hph1* genes, mRNA, or protein can be made as described above. Underexpression of the *hph1* gene in the tissue suspected of being neoplastic indicates neoplasia in the tissue. The degree of underexpression of the *hph1* gene in the neoplastic tissue relative to wild-type expression of the gene in normal tissue, or differences in the amount of underexpression of the *hph1* gene in the neoplastic tissue over time, can be used to prognose the progression of the neoplasia



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in that tissue or to monitor the response of the neoplastic tissue to various therapeutic regimens over time.

5 In addition, a genetic predisposition to neoplasia in a human can be detected by comparing a wild-type *hph1* gene, mRNA, or protein with an *hph1* gene, mRNA, or protein in a fetal tissue. Fetal tissues which can be used for this purpose include, but are not limited to, amniotic fluid, chorionic villi, blood, and the blastomere of an *in vitro*-fertilized embryo. The wild-type *hph1* gene can be obtained from any tissue. The mRNA or protein can be obtained from a normal tissue of a human in which the *hph1* gene is expressed. Such tissues are disclosed above. Differences such as  
10 alterations in the nucleotide sequence or size of the fetal *hph1* gene or mRNA, or alterations in the molecular weight, amino acid sequence, or relative abundance of fetal *hph1* protein indicate a germline mutation in the *hph1* gene of the fetus which indicates a genetic predisposition to neoplasia.

15 The invention provides a therapeutic composition for restoring a wild-type *hph1* function to a cell which has lost that function. Wild-type *hph1* functions include suppression of neoplasia and of dysplastic or hyperplastic cell growth, as well as the ability to induce cellular differentiation. The cell can be any cell of a human which would normally express the wild-type *hph1* gene, such as thymus, testis, heart, prostate, ovary, small intestine, peripheral blood lymphocytes, skeletal muscle,  
20 pancreas, lung, liver, and kidney, but which lacks *hph1* expression or expresses an altered form of *hph1* mRNA or protein. Such cells include neoplasias of the tissues mentioned above as well as any other neoplastic cells which have lost wild-type polyhomeotic 1 function. The therapeutic composition comprises all or a portion of a wild-type human *hph1* gene or gene expression product in a pharmaceutically acceptable carrier. The *hph1* expression product can be, e.g., mRNA or protein. A  
25 portion of a wild-type *hph1* gene, mRNA, or protein can also be used if it is capable of restoring wild-type *hph1* function to the cell.

30 Pharmaceutically acceptable carriers are well known to those in the art. Such carriers include, but are not limited to, large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Pharmaceutically acceptable

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salts can also be used in the composition, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as the salts of organic acids such as acetates, propionates, malonates, or benzoates. The composition can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances  
5 such as wetting agents, emulsifying agents, or pH buffering agents. Liposomes, such as those described in U.S. 5,422,120, WO 95/13796, WO 91/14445, or EP 524,968 B1, can also be used as a carrier for the therapeutic polyhomeotic 1 composition.

Typically, the therapeutic *hph1* composition is prepared as an injectable, either as a liquid solution or suspension, however solid forms suitable for solution in, or  
10 suspension in, liquid vehicles prior to injection can also be prepared. The composition can also be formulated into an enteric coated tablet or gel capsule according to known methods in the art, such as those described in U.S. 4,853,230, EP 225,189, AU 9,224,296, and AU 9,230,801.

Proliferative disorders, such as neoplasias, dysplasias, and hyperplasias, can be  
15 treated by administration of the therapeutic *hph1* composition. Neoplasias which can be treated with the therapeutic composition include, but are not limited to, melanomas, squamous cell carcinomas, adenocarcinomas, hepatocellular carcinomas, renal cell carcinomas, sarcomas, myosarcomas, non-small cell lung carcinomas, leukemias, lymphomas, osteosarcomas, central nervous system tumors such as gliomas,  
20 astrocytomas, oligodendrogliomas, and neuroblastomas, tumors of mixed origin, such as Wilms' tumor and teratocarcinomas, and metastatic tumors. Proliferative disorders which can be treated with the therapeutic composition include disorders such as anhydric hereditary ectodermal dysplasia, congenital alveolar dysplasia, epithelial dysplasia of the cervix, fibrous dysplasia of bone, and mammary dysplasia.  
25 Hyperplasias, for example, endometrial, adrenal, breast, prostate, or thyroid hyperplasias or pseudoepitheliomatous hyperplasia of the skin can be treated with wild-type *hph1* therapeutic compositions. Even in disorders in which *hph1* mutations are not implicated, upregulation or enhancement of *hph1* function can have therapeutic application. In these disorders, increasing *hph1* expression or enhancing *hph1* function  
30 can help to suppress tumors. Similarly, in tumors where *hph1* expression is not

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aberrant, effecting *hph1* upregulation or augmentation of *hph1* activity can suppress metastases.

Both the dose of the *hph1* composition and the means of administration can be determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. If the composition contains *hph1* protein or polypeptide, effective dosages of the composition are in the range of about 5  $\mu$ g to about 50  $\mu$ g/kg of patient body weight, also about 50  $\mu$ g to about 5 mg/kg, also about 100  $\mu$ g to about 500  $\mu$ g/kg of patient body weight, and about 200 to about 250  $\mu$ g/kg. Administration of the therapeutic agents of the invention can include local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration.

Alternatively, the therapeutic composition can contain *hph1* polynucleotides. Preferably, the therapeutic composition contains an expression construct comprising a promoter and a polynucleotide segment encoding at least eight contiguous amino acids of *hph1* protein. Within the expression construct, the polynucleotide segment is located downstream from the promoter, and transcription of the polynucleotide segment initiates at the promoter. Various methods can be used to administer the therapeutic composition directly to a specific site in the body. For example, a small metastatic lesion can be located and the therapeutic composition injected several times in several different locations within the body of tumor. Alternatively, arteries which serve a tumor can be identified, and the therapeutic composition injected into such an artery, in order to deliver the composition directly into the tumor. A tumor which has a necrotic center can be aspirated and the composition injected directly into the now empty center of the tumor. The *hph1* composition can be directly administered to the surface of the tumor, for example, by topical application of the composition. X-ray imaging can be used to assist in certain of the above delivery methods.

Additionally, combination therapeutic agents including an *hph1* protein or polypeptide or a subgenomic *hph1* polynucleotide and other therapeutic agents can be administered together. The co-administration can be simultaneous, achieved for example by placing polynucleotides encoding the agents in the same expression

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construct, or by putting the agents, whether polynucleotide, polypeptide, or other drug, in the same pharmaceutical composition, or by administering the agents in different pharmaceutical compositions injected at about the same time in the same location. If the co-administration is not simultaneous, for example, in the case of administration of a prodrug after administration of a prodrug activator, the second agent can be administered by direct injection as appropriate for the goals of the therapy. Thus, for example, in the case of an administration of a prodrug, the prodrug is administered at the same location as the prodrug activator. Thus, a co-administration protocol can include a combination of administrations to achieve the goal of the therapy. The co-administration can include subsequent administrations as is necessary.

Receptor-mediated targeted delivery of therapeutic compositions containing *hphI* subgenomic polynucleotides to specific tissues can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al. (1993), *Trends in Biotechnol.* 11, 202-05; Chiou et al. (1994), GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.); Wu & Wu (1988), *J. Biol. Chem.* 263, 621-24; Wu et al. (1994), *J. Biol. Chem.* 269, 542-46; Zenke et al. (1990), *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59; Wu et al. (1991), *J. Biol. Chem.* 266, 338-42.

Alternatively, the composition containing subgenomic *hphI* polynucleotides can be introduced into human cells *ex vivo* and then replaced into the human. Cells can be removed from a variety of locations including, for example, from a selected tumor or from an affected organ. In addition, the therapeutic composition can be inserted into non-tumorigenic cells, for example, dermal fibroblasts or peripheral blood leukocytes. If desired, particular fractions of cells such as a T cell subset or stem cells can also be specifically removed from the blood (*see*, for example, PCT WO 91/16116). The *hphI*-containing therapeutic composition can then be contacted with the removed cells utilizing any of the above-described techniques, followed by the return of the cells to the human, preferably to or within the vicinity of a tumor. The above-described methods can additionally comprise the steps of depleting fibroblasts or other non-contaminating tumor cells subsequent to removing tumor cells from a human, and/or the step of inactivating the cells, for example, by irradiation.

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Therapeutic compositions containing *hphI* subgenomic polynucleotides can be administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA can also be used during a gene therapy protocol. Factors such as method of action and efficacy of transformation and expression are considerations that will effect the dosage required for ultimate efficacy of the *hphI* subgenomic polynucleotides. Where greater expression is desired over a larger area of tissue, larger amounts of *hphI* subgenomic polynucleotides or the same amounts readministered in a successive protocol of administrations, or several administrations to different adjacent or close tissue portions of for example, a tumor site, may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect.

The *hphI* therapeutic composition can also be used to induce differentiation of a progenitor cell, *e.g.*, in order to study the process of differentiation and test compounds which affect this process. Induction of differentiation is also desirable, for example, in the treatment of anaplastic tumors, which are composed of cells which have lost some of their differentiated characteristics. Progenitor cells which can be induced to differentiate using an *hphI* therapeutic composition comprising *hphI* subgenomic polynucleotides, proteins, polypeptides, or fusion proteins include, but are not limited to, erythropoietic stem cells, neuroblasts, chondroblasts, melanoblasts, myoblasts, and neural crest cells.

In addition to methods using the *hphI* therapeutic composition described above, expression of an endogenous *hphI* gene in a cell can be altered by introducing in frame with the endogenous *hphI* gene a DNA construct comprising an *hphI* targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site by homologous recombination, such that a homologously recombinant cell comprising the DNA construct is formed. This method of affecting endogenous gene expression is taught in U.S. Patent No. 5,641,670, which is incorporated herein in its entirety by reference.

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The targeting sequence is selected from the nucleotide sequence shown in SEQ ID NO:1. The transcription unit is located upstream of a coding sequence of the endogenous *hphI* gene. The exogenous regulatory sequence directs transcription of the coding sequence of the *hphI* gene. Preferably, the homologously recombinant cell is a progenitor cell or a neoplastic cell, and the exogenous regulatory sequence directs enhanced transcription of the coding sequence of the *hphI* gene.

Cells transformed with wild-type *hphI* subgenomic polynucleotides can be used as model systems to study cancer remission and drug treatments which promote such remission. Suppression of the neoplastic phenotype is a process which involves alterations in gene expression in the transformed cells. These alterations in gene expression will be reflected in morphological and biochemical changes in the transformed cells. Morphological changes can be studied, for example, by observing the transformed cells microscopically and comparing the appearance of the transformed cells with cells which have not received a wild-type *hphI* gene. Biochemical alterations can be studied, *inter alia*, by comparing the proteins which are expressed by the cells before and at various times after transformation with the wild-type *hphI* gene. Methods of comparing proteins between two cells, such as using SDS polyacrylamide electrophoresis, are well known in the art. Cells transformed with a wild-type *hphI* gene and in the process of suppressing neoplastic growth can also be exposed to various drug treatments to determine which treatments promote the morphological or biochemical changes which accompany suppression of the neoplastic phenotype. Similarly, cells transformed with wild-type *hphI* subgenomic polynucleotides can also be used to study the changes which accompany cellular differentiation in progenitor cells and the response of these cells to test compounds which affect differentiation.

A more complete description of gene therapy vectors, especially retroviral vectors is contained in U.S. Serial No. 08/869,309, which is expressly incorporated herein.

**SYNOPSIS OF THE INVENTION**

1. An isolated and purified human polyhomeotic 1 protein having the amino acid sequence shown in SEQ ID NO:2.

2. An isolated and purified human polyhomeotic 1 polypeptide consisting of at least 22 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2.

3. An *hph1* fusion protein comprising a first protein segment and a second protein segment fused together by means of a peptide bond, wherein the first protein segment consists of at least 8 contiguous amino acids of a human polyhomeotic 1 protein.

4. A preparation of antibodies which specifically bind to a human polyhomeotic 1 protein.

5. The preparation of antibodies of item 4 wherein the antibodies are monoclonal.

6. The preparation of antibodies of item 4 wherein the antibodies are polyclonal.

7. An isolated and purified subgenomic polynucleotide consisting of at least 10 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1.

8. An expression construct for expressing all or a portion of a human polyhomeotic 1 protein comprising:

a promoter; and

a polynucleotide segment encoding at least 8 contiguous amino acids of a human polyhomeotic 1 protein, wherein the polynucleotide segment is located downstream from the promoter, wherein transcription of the polynucleotide segment initiates at the promoter.

9. A host cell comprising the expression construct of item 8.

10. A homologously recombinant cell having incorporated therein a new transcription initiation unit, wherein the new transcription initiation unit comprises:

(a) an exogenous regulatory sequence;

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(b) an exogenous exon; and

(c) a splice donor site, wherein the transcription initiation unit is located upstream of a coding sequence of an *hphI* gene, wherein the exogenous regulatory sequence directs transcription of the coding sequence of the *hphI* gene.

5           11.     The homologously recombinant cell of item 10 wherein the exogenous regulatory sequence directs enhanced transcription of the coding sequence of the *hphI* gene.

          12.     The homologously recombinant cell of item 10 wherein the homologously recombinant cell is a neoplastic cell.

10           13.     The homologously recombinant cell of item 10 wherein the homologously recombinant cell is a progenitor cell.

          14.     A method of identifying neoplastic tissue of a human, comprising:  
                  comparing the expression of a polyhomeotic 1 gene in a first tissue of  
a human suspected of being neoplastic with the expression of a polyhomeotic 1 gene  
15     in a second tissue of the human which is normal, wherein underexpression of the  
human polyhomeotic 1 gene in the first tissue identifies the first tissue as being  
neoplastic.

          15.     The method of item 14 wherein the polyhomeotic 1 genes in the first  
and second tissues are compared by sequencing the polyhomeotic 1 genes in the first  
20     and second tissues.

          16.     The method of item 14 wherein the difference in the polyhomeotic 1  
gene between the first and second tissues is detected by amplification of the  
polyhomeotic 1 genes of the first and second tissues and hybridization of the amplified  
genes to nucleotide probes.

25           17.     The method of item 14 wherein polyhomeotic 1 mRNA is compared  
between the first and second tissues.

          18.     The method of item 14 wherein a polyhomeotic 1 protein is compared  
between the first and second tissues.

30           19.     A method to aid in the diagnosis or prognosis of neoplasia in a human,  
comprising:



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comparing a polyhomeotic 1 gene, mRNA, or protein in a first tissue of a human suspected of being neoplastic with a polyhomeotic 1 gene, mRNA, or protein in a second tissue of a human which is normal, wherein a difference between the polyhomeotic 1 genes, mRNAs, or proteins in the first and second tissues indicates neoplasia in the first tissue.

20. The method of item 19 wherein the polyhomeotic 1 genes in the first and second tissues are compared by sequencing the polyhomeotic 1 genes in the first and second tissues.

21. The method of item 19 wherein the difference in the polyhomeotic 1 gene between the first and second tissues is detected by amplification of the polyhomeotic 1 genes of the first and second tissues and hybridization of the amplified genes to nucleotide probes.

22. The method of item 19 wherein polyhomeotic mRNA is compared.

23. The method of item 19 wherein polyhomeotic protein is compared.

24. A method to aid in detecting a genetic predisposition to neoplasia in a human, comprising:

comparing a polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of a human with a wild-type human polyhomeotic 1 gene, mRNA, or protein, wherein a difference between the polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of the human and the wild-type human polyhomeotic 1 gene, mRNA, or protein indicates a genetic predisposition to neoplasia in the human.

25. The method of item 24 wherein a polyhomeotic 1 mRNA is compared.

26. The method of item 24 wherein a polyhomeotic 1 gene is compared.

27. The method of item 24 wherein the difference between the polyhomeotic 1 genes is detected by amplification of the wild-type human polyhomeotic 1 gene and the human polyhomeotic 1 gene in the fetal tissue and hybridizing the amplified genes to nucleotide probes.

28. The method of item 24 wherein a human polyhomeotic 1 protein is compared.

29. A method of identifying a human chromosome 12, comprising the steps of:

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contacting a preparation of metaphase human chromosomes with a nucleotide probe comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1; and

5 detecting a region of a chromosome which specifically hybridizes to the nucleotide probe, wherein a region of a chromosome which specifically hybridizes to the nucleotide probe is identified as a human chromosome 12.

30. The method of item 29 wherein the region of the chromosome which specifically hybridizes to the nucleotide probe is identified as the short arm of a human chromosome 12.

10 31. The method of item 29 wherein the region of the chromosome which specifically hybridizes to the nucleotide probe is identified as a p13 region of a human chromosome 12.

32. A therapeutic composition for restoring a wild-type human polyhomeotic 1 function to a cell which lacks that function, comprising:

15 all or a portion of a wild-type human polyhomeotic 1 gene or expression product, wherein said all or a portion of the wild-type human polyhomeotic 1 gene or expression product is capable of restoring a wild-type polyhomeotic 1 function to a cell; and

a pharmaceutically acceptable carrier.

20 33. A method of treating proliferative disorders, comprising the step of administering to a human a composition comprising all or a portion of a wild-type human polyhomeotic 1 gene or expression product, wherein said all or a portion of the wild-type human polyhomeotic 1 gene or expression is capable of restoring or enhancing a wild-type polyhomeotic 1 function to a cell.

25 34. A method of inducing a cell to differentiate, comprising the step of contacting a progenitor cell with a composition comprising all or a portion of a human polyhomeotic 1 gene or expression product, wherein said all or a portion of the human polyhomeotic 1 gene or expression product is capable of inducing differentiation of the cell.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (I) APPLICANT: RANDAZZO, FILIPPO
- (ii) TITLE OF INVENTION: HUMAN POLYHOMEOTIC 1 (hph1) ACTS AS A TUMOR SUPPRESSOR
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: CHIRON CORPORATION
  - (B) STREET: 4560 HORTON STREET
  - (C) CITY: EMERYVILLE
  - (D) STATE: CA
  - (E) COUNTRY: USA
  - (F) ZIP: 94608
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: POTTERFIRESTONE, LEIGH H.
  - (B) REGISTRATION NUMBER: 36,831
  - (C) REFERENCE/DOCKET NUMBER: 1355.
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 510-923-2707
  - (B) TELEFAX: 510-655-3542

## (2) INFORMATION FOR SEQ ID NO:1:

- (I) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3879 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGAGACTG AGAGCGAGCA GAACTCCAAT TCCACCAATG GGAGTTCTAG CTCAGGGGGC 60  
AGCTCTCGGC CCCAGATAGC TCAAATGTCA CTATATGAAC GACAAGCAGT GCAGGCTCTG 120

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CAAGCACTGC AGCGGCAGCC CAATGCAGCT CAGTATTTCC ACCAGTTCAT GCTCCAGCAG	180
CAGCTCAGTA ATGCCCAGCT GCATAGCCTG GCTGCCGTCC AGCAGGCCAC AATTGCTGCC	240
AGTCGGCAGG CCAGCTCCCC AAACACCAGC ACTACACAGC AGCAGACTAC CACCACCCAG	300
GCCTCGATCA ATCTGGCCAC CACATCGGCC GCCCAGCTCA TCAGCCGATC CCAGAGTGTG	360
AGCTCTCCTA GTGCTACCAC CTTGACCCAA TCTGTGCTAC TGGGGAACAC CACCTCCCCA	420
CCCCTCAACC AGTCTCAGGC CCAGATGTAT CTACGGCCAC AGCTGGGAAA CCTATTGCAG	480
GTAAACCGAA CCCTGGGTCG GAATGTGCCT CTAGCCTCCC AACTCATCCT GATGCCTAAT	540
GGGGCGGTGG CTGCAGTCCA GCAGGAGGTG CCATCTGCTC AGTCTCCTGG AGTTCATGCA	600
GATGCAGATC AGGTTTCAGAA CTTGGCAGTA AGGAATCAAC AGGCCTCAGC TCAAGGACCT	660
CAGATGCAAG GCTCCACTCA GAAGGCCATT CCTCCAGGAG CCTCCCCTGT CTCTAGCCTC	720
TCCCAGGCCT CTAGCCAGGC CCTAGCGGTG GCACAGGCTT CCTCTGGGGC CACAAACCAG	780
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GGTGGGAGCT GTCCCAGGAA GGTACAGGA GTGGTGCAGC CCTTGCCTGC AGCCCAAACA	960
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GCAGATGGGA GTGGCCAGCA GAATGTGGGC ATGAACCTGA CACGGACAGC CACACCTGCG	1080
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GCTGCCTTCT ATATGCAGTC TGTGCACTTG CCGGGTAAAC CCCAGACATT GGCTGTCAAA	1920
CGCAAGGCTG ACTCTGAGGA GGAGAGAGAT GATGTCTCCA CATTGGGTTC AATGCTTCCT	1980
GCCAAAGCAT CTCCAGTAGC AGAAAGCCCA AAAGTCATGG ACGAGAAGAG CAGTCTTGGA	2040
GAAAAAGCTG AATCAGTGGC TAATGTGAAT GCTAATACTC CAAGCAGTGA ACTAGTAGCC	2100

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TTGACCCCCG CCCCTTCAGT ACCGCCTCCT ACACTAGCCA TGGTGTCTAG ACAAATGGGT 2160  
GACTCAAAAC CCCACAGGC CATCGTGAAG CCCAGATTC TCACCCACAT CATTGAAGGC 2220  
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TCTGAGAAGC CACTACAGAC TGGCCTTCCG ACAGGGCTGA CTGAGAATCA GTCAGGTGGC 2340  
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GAGTACTGTG GGAAGTACGC CCCCGCAGAG CAGTTTCGTG GCTCTAAGAG GTTCTGCTCC 2460  
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CTCAAGGAGA CCTAAGGTGG CCTCTTGCA CAAACCAGCC TAAGGCAGAC ACTCTCCACT 3060  
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GTAACCTTCT GTAGGGGATT ACTGAGACAG GGAAGAGAAG TGCAAGAATT GGTTGCTGGT 3180  
GCTACATGGC GGCAGCTTG ACATTTTCTC TGGGTTCTAC TTTATTTTTT AAAATCTTTA 3240  
CAGTTCTCAC CATTTACGT ACCTTAATCC AATCTTTATA AAAGAGGCAG TCTAGAGAAC 3300  
TAGGACTGCT CAGCCTTATC CTGGAGTGA GCATTTAGCC CAGGTCTTAA TTCTCCAAGA 3360  
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TCTTCTGCTG CAGGGACTAA AAGTATTGTA CTGGGGCACA TGTGGCTGTT GTCATTCTTT 3660  
CTGCATCCCA CTGTTCCCCT CCAATTTATG TTATTTTCTA CCCTGTTTTT CAGTTCCATC 3720  
TCTGCTCTGT CCTATAGCTT TATAAAACCA GAGTGTGTGG GGCTGAGGTC AGGAGTATAA 3780  
GTACCTGCCT TAGGCACTAT TCCTTATATA ACAAAAATAT TAAATATTTT TTTCTCAGT 3840  
AAAAGGATGA AAATTGGTAA AAAAAAAAAA AAAAAAAGG 3879

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1004 amino acids
- (B) TYPE: amino acid

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(C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Glu	Thr	Glu	Ser	Glu	Gln	Asn	Ser	Asn	Ser	Thr	Asn	Gly	Ser	Ser	1	5	10	15
Ser	Ser	Gly	Gly	Ser	Ser	Arg	Pro	Gln	Ile	Ala	Gln	Met	Ser	Leu	Tyr	20	25	30	
Glu	Arg	Gln	Ala	Val	Gln	Ala	Leu	Gln	Ala	Leu	Gln	Arg	Gln	Pro	Asn	35	40	45	
Ala	Ala	Gln	Tyr	Phe	His	Gln	Phe	Met	Leu	Gln	Gln	Gln	Leu	Ser	Asn	50	55	60	
Ala	Gln	Leu	His	Ser	Leu	Ala	Ala	Val	Gln	Gln	Ala	Thr	Ile	Ala	Ala	65	70	75	80
Ser	Arg	Gln	Ala	Ser	Ser	Pro	Asn	Thr	Ser	Thr	Thr	Gln	Gln	Gln	Thr	85	90	95	
Thr	Thr	Thr	Gln	Ala	Ser	Ile	Asn	Leu	Ala	Thr	Thr	Ser	Ala	Ala	Gln	100	105	110	
Leu	Ile	Ser	Arg	Ser	Gln	Ser	Val	Ser	Ser	Pro	Ser	Ala	Thr	Thr	Leu	115	120	125	
Thr	Gln	Ser	Val	Leu	Leu	Gly	Asn	Thr	Thr	Ser	Pro	Pro	Leu	Asn	Gln	130	135	140	
Ser	Gln	Ala	Gln	Met	Tyr	Leu	Arg	Pro	Gln	Leu	Gly	Asn	Leu	Leu	Gln	145	150	155	160
Val	Asn	Arg	Thr	Leu	Gly	Arg	Asn	Val	Pro	Leu	Ala	Ser	Gln	Leu	Ile	165	170	175	
Leu	Met	Pro	Asn	Gly	Ala	Val	Ala	Ala	Val	Gln	Gln	Glu	Val	Pro	Ser	180	185	190	
Ala	Gln	Ser	Pro	Gly	Val	His	Ala	Asp	Ala	Asp	Gln	Val	Gln	Asn	Leu	195	200	205	
Ala	Val	Arg	Asn	Gln	Gln	Ala	Ser	Ala	Gln	Gly	Pro	Gln	Met	Gln	Gly	210	215	220	
Ser	Thr	Gln	Lys	Ala	Ile	Pro	Pro	Gly	Ala	Ser	Pro	Val	Ser	Ser	Leu	225	230	235	240
Ser	Gln	Ala	Ser	Ser	Gln	Ala	Leu	Ala	Val	Ala	Gln	Ala	Ser	Ser	Gly	245	250	255	
Ala	Thr	Asn	Gln	Ser	Leu	Asn	Leu	Ser	Gln	Ala	Gly	Gly	Gly	Ser	Gly	260	265	270	
Asn	Ser	Ile	Pro	Gly	Ser	Met	Gly	Pro	Gly	Gly	Gly	Gly	Gln	Ala	His	275	280	285	
Gly	Gly	Leu	Gly	Gln	Leu	Pro	Ser	Ser	Gly	Met	Gly	Gly	Gly	Ser	Cys	290	295	300	
Pro	Arg	Lys	Gly	Thr	Gly	Val	Val	Gln	Pro	Leu	Pro	Ala	Ala	Gln	Thr	305	310	315	320
Val	Thr	Val	Ser	Gln	Gly	Ser	Gln	Thr	Glu	Ala	Glu	Ser	Ala	Ala	Ala	325	330	335	
Lys	Lys	Ala	Glu	Ala	Asp	Gly	Ser	Gly	Gln	Gln	Asn	Val	Gly	Met	Asn	340	345	350	
Leu	Thr	Arg	Thr	Ala	Thr	Pro	Ala	Pro	Ser	Gln	Thr	Leu	Ile	Ser	Ser	355	360	365	
Ala	Thr	Tyr	Thr	Gln	Ile	Gln	Pro	His	Ser	Leu	Ile	Gln	Gln	Gln	Gln	370	375	380	
Gln	Ile	His	Leu	Gln	Gln	Lys	Gln	Val	Val	Ile	Gln	Gln	Gln	Ile	Ala	385	390	395	400
Ile	His	His	Gln	Gln	Gln	Phe	Gln	His	Arg	Gln	Ser	Gln	Leu	Leu	His				

										405					410					415				
Thr	Ala	Thr	His	Leu	Gln	Leu	Ala	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln					
				420					425					430										
Gln	Gln	Gln	Gln	Gln	Gln	Gln	Pro	Gln	Ala	Thr	Thr	Leu	Thr	Ala	Pro									
				435					440					445										
Gln	Pro	Pro	Gln	Val	Pro	Pro	Thr	Gln	Gln	Val	Pro	Pro	Ser	Gln	Ser									
				450					455					460										
Gln	Gln	Gln	Ala	Gln	Thr	Leu	Val	Val	Gln	Pro	Met	Leu	Gln	Ser	Ser									
				465					470					475										
				485					490					495										
Pro	Leu	Ser	Leu	Pro	Pro	Asp	Ala	Ala	Pro	Lys	Pro	Pro	Ile	Pro	Ile									
				500					505					510										
Gln	Ser	Lys	Pro	Pro	Val	Ala	Pro	Ile	Lys	Pro	Pro	Gln	Leu	Gly	Ala									
				515					520					525										
Ala	Lys	Met	Ser	Ala	Ala	Gln	Gln	Pro	Pro	His	Ile	Pro	Val	Gln										
				530					535					540										
Val	Val	Gly	Thr	Arg	Gln	Pro	Gly	Thr	Ala	Gln	Ala	Gln	Ala	Leu	Gly									
				545					550					555										
Leu	Ala	Gln	Leu	Ala	Ala	Ala	Val	Pro	Thr	Ser	Arg	Gly	Met	Pro	Gly									
				565					570					575										
Thr	Val	Gln	Ser	Gly	Gln	Ala	His	Leu	Ala	Ser	Ser	Pro	Pro	Ser	Ser									
				580					585					590										
Gln	Ala	Pro	Gly	Ala	Leu	Gln	Glu	Cys	Pro	Pro	Thr	Leu	Ala	Pro	Gly									
				595					600					605										
Met	Thr	Leu	Ala	Pro	Val	Gln	Gly	Thr	Ala	His	Val	Val	Lys	Gly	Gly									
				610					615					620										
Ala	Thr	Thr	Ser	Ser	Pro	Val	Val	Ala	Gln	Val	Pro	Ala	Ala	Phe	Tyr									
				625					630					635										
Met	Gln	Ser	Val	His	Leu	Pro	Gly	Lys	Pro	Gln	Thr	Leu	Ala	Val	Lys									
				645					650					655										
Arg	Lys	Ala	Asp	Ser	Glu	Glu	Glu	Arg	Asp	Asp	Val	Ser	Thr	Leu	Gly									
				660					665					670										
Ser	Met	Leu	Pro	Ala	Lys	Ala	Ser	Pro	Val	Ala	Glu	Ser	Pro	Lys	Val									
				675					680					685										
Met	Asp	Glu	Lys	Ser	Ser	Leu	Gly	Glu	Lys	Ala	Glu	Ser	Val	Ala	Asn									
				690					695					700										
Val	Asn	Ala	Asn	Thr	Pro	Ser	Ser	Glu	Leu	Val	Ala	Leu	Thr	Pro	Ala									
				705					710					715										
Pro	Ser	Val	Pro	Pro	Pro	Thr	Leu	Ala	Met	Val	Ser	Arg	Gln	Met	Gly									
				725					730					735										
Asp	Ser	Lys	Pro	Pro	Gln	Ala	Ile	Val	Lys	Pro	Gln	Ile	Leu	Thr	His									
				740					745					750										
Ile	Ile	Glu	Gly	Phe	Val	Ile	Gln	Glu	Gly	Ala	Glu	Pro	Phe	Pro	Val									
				755					760					765										
Gly	Cys	Ser	Gln	Leu	Leu	Lys	Glu	Ser	Glu	Lys	Pro	Leu	Gln	Thr	Gly									
				770					775					780										
Leu	Pro	Thr	Gly	Leu	Thr	Glu	Asn	Gln	Ser	Gly	Gly	Pro	Leu	Gly	Val									
				785					790					795										
Asp	Ser	Pro	Ser	Ala	Glu	Leu	Asp	Lys	Lys	Ala	Asn	Leu	Leu	Lys	Cys									
				805					810															

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Ser	Ser	Arg	Gly	Ser 885	Asp	Asn	Ser	Ser	Tyr 890	Asp	Glu	Ala	Leu	Ser 895	Pro
Thr	Ser	Pro	Gly 900	Pro	Leu	Ser	Val	Arg 905	Ala	Gly	His	Gly	Glu 910	Arg	Asp
Leu	Gly	Asn 915	Pro	Asn	Thr	Ala	Pro 920	Pro	Thr	Pro	Glu	Leu 925	His	Gly	Ile
Asn	Pro	Val	Phe	Leu	Ser	Ser 935	Asn	Pro	Ser	Arg	Trp 940	Ser	Val	Glu	Glu
Val 945	Tyr	Glu	Phe	Ile	Ala 950	Ser	Leu	Gln	Gly	Cys 955	Gln	Glu	Ile	Ala	Glu 960
Glu	Phe	Arg	Ser	Gln 965	Glu	Ile	Asp	Gly	Gln 970	Ala	Leu	Leu	Leu 975	Leu	Lys
Glu	Glu	His	Leu 980	Met	Ser	Ala	Met	Asn 985	Ile	Lys	Leu	Gly	Pro 990	Ala	Leu
Lys	Ile	Cys 995	Ala	Lys	Ile	Asn	Val 1000	Leu	Lys	Glu	Thr				



**CLAIMS**

1. An isolated and purified human polyhomeotic 1 protein having the amino acid sequence shown in SEQ ID NO:2.

2. An isolated and purified human polyhomeotic 1 polypeptide consisting of at least 22 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2.

3. An *hph1* fusion protein comprising a first protein segment and a second protein segment fused together by means of a peptide bond, wherein the first protein segment consists of at least 8 contiguous amino acids of a human polyhomeotic 1 protein.

4. A preparation of antibodies which specifically bind to a human polyhomeotic 1 protein.

5. An isolated and purified subgenomic polynucleotide consisting of at least 10 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1.

6. An expression construct for expressing all or a portion of a human polyhomeotic 1 protein comprising:

a promoter; and

a polynucleotide segment encoding at least 8 contiguous amino acids of a human polyhomeotic 1 protein, wherein the polynucleotide segment is located downstream from the promoter, wherein transcription of the polynucleotide segment initiates at the promoter.

7. A homologously recombinant cell having incorporated therein a new transcription initiation unit, wherein the new transcription initiation unit comprises:

(a) an exogenous regulatory sequence;

(b) an exogenous exon; and

(c) a splice donor site, wherein the transcription initiation unit is located upstream of a coding sequence of an *hph1* gene, wherein the exogenous regulatory sequence directs transcription of the coding sequence of the *hph1* gene.

8. A method of identifying neoplastic tissue of a human, comprising:

comparing the expression of a polyhomeotic 1 gene in a first tissue of a human suspected of being neoplastic with the expression of a polyhomeotic 1 gene in a second tissue of the human which is normal, wherein underexpression of the human polyhomeotic 1 gene in the first tissue identifies the first tissue as being neoplastic.

9. A method to aid in the diagnosis or prognosis of neoplasia in a human, comprising:

comparing a polyhomeotic 1 gene, mRNA, or protein in a first tissue of a human suspected of being neoplastic with a polyhomeotic 1 gene, mRNA, or protein in a second tissue of a human which is normal, wherein a difference between the polyhomeotic 1 genes, mRNAs, or proteins in the first and second tissues indicates neoplasia in the first tissue.

10. A method to aid in detecting a genetic predisposition to neoplasia in a human, comprising:

comparing a polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of a human with a wild-type human polyhomeotic 1 gene, mRNA, or protein, wherein a difference between the polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of the human and the wild-type human polyhomeotic 1 gene, mRNA, or protein indicates a genetic predisposition to neoplasia in the human.

11. A method of identifying a human chromosome 12, comprising the steps of:

contacting a preparation of metaphase human chromosomes with a nucleotide probe comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1; and

detecting a chromosome which specifically hybridizes to the nucleotide probe, wherein a chromosome which specifically hybridizes to the nucleotide probe is identified as a human chromosome 12.

12. A therapeutic composition for restoring a wild-type human polyhomeotic 1 function to a cell which lacks that function, comprising:

all or a portion of a wild-type human polyhomeotic 1 gene or expression product, wherein said all or a portion of the wild-type human polyhomeotic 1 gene or expression product is capable of restoring a wild-type polyhomeotic 1 function to a cell; and

5 a pharmaceutically acceptable carrier.

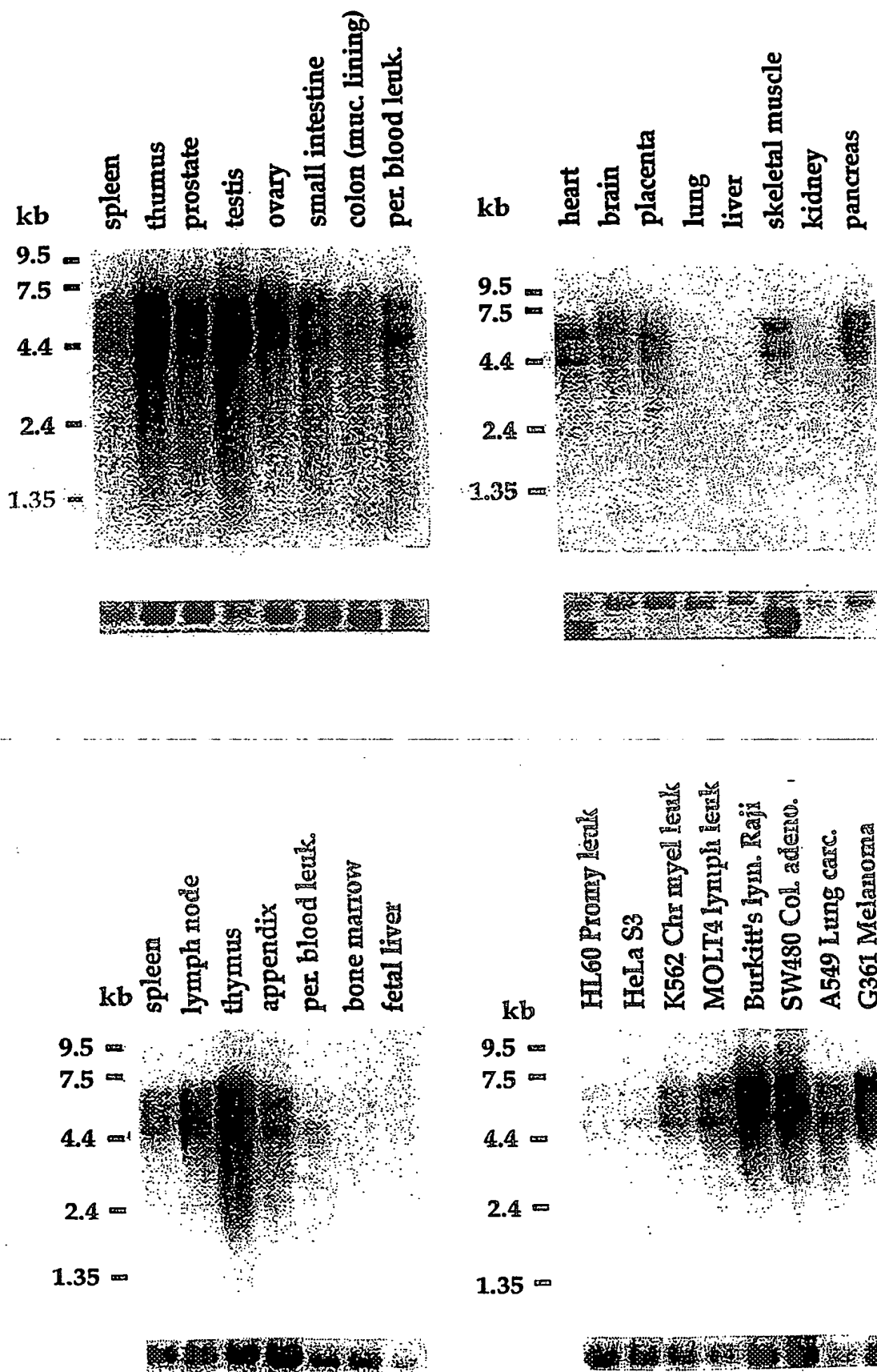
13. A method of treating proliferative disorders, comprising the step of administering to a human a composition comprising all or a portion of a wild-type human polyhomeotic 1 gene or expression product, wherein said all or a portion of the wild-type human polyhomeotic 1 gene or expression is capable of restoring or enhancing a wild-type polyhomeotic 1 function to a cell.

14. A method of inducing a cell to differentiate, comprising the step of contacting a progenitor cell with a composition comprising all or a portion of a human polyhomeotic 1 gene or expression product, wherein said all or a portion of the human polyhomeotic 1 gene or expression product is capable of inducing differentiation of the cell.

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**FIG. 1**

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 97/14866

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/82 C07K16/32 C07K16/18 C07K19/00  
C12Q1/68 A61K48/00 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NOMURA, M. ET AL.: "Isolation and characterization of retinoic acid-inducible cDNA clones in F9 cells: one of the early inducible clones encodes a novel protein sharing several highly homologous regions with a Drosophila polyhomeotic protein" DIFFERENTIATION, vol. 57, 1994, pages 39-50, XP002046787 see the whole document	2,3,6
X	DATABASE EMBL EMBEST2, RELEASE 44 Human EST AC No. R77179, 10 May 1995 HILLIER, J. ET AL.: "The WashU-Werck EST Project" XP002046790 see abstract	5

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

13 November 1997

Date of mailing of the international search report

27.11.97

Name and mailing address of the ISA

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Authorized officer

Smalt, R

# INTERNATIONAL SEARCH REPORT

Inter. Appl. No.

PCT/US 97/14866

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE EMBL EMSTS, RELEASE 47  Human STS, AC. No. X97939, 27 May 1996  PUJANA, M.A. ET AL.: "Cloning (CAG/GTC)n  STSs by an Alu-(CAG/GTC)n PCR method: an  approach to human chromosome 12 and  spinocerebellar ataxia 2 (SCA2)"  XP002046791  see abstract</p> <p style="text-align: center;">---</p>	11
Y	<p>DATABASE WPI  Derwent Publications Ltd., London, GB;  AN 92-123644  XP002046792  CHEPELINSK, A.B. ET AL.: "Major intrinsic  protein gene prod. - used to detect  cataracts and DNA encoding it used as  probe for human genome " (US DEPT. HEALTH  &amp; HUMAN SERVICE) , 25 February 1992  see abstract</p> <p style="text-align: center;">---</p>	11
P,X	<p>GUNSTER, M.J. ET AL.: "Identification and  characterization of interactions between  the vertebrate polycomb-group protein BMI1  and human homologs of polyhomeotic "  MOLECULAR AND CELLULAR BIOLOGY,  vol. 17, no. 4, April 1997,  pages 2326-2335, XP002046788  see the whole document</p> <p style="text-align: center;">---</p>	2-5
P,X	<p>NUCLEIC ACIDS RESEARCH,  vol. 24, no. 18, 15 September 1996,  XP002046789  see the whole document</p> <p style="text-align: center;">---</p>	11
A	<p>WO 95 31560 A (TRANSKARYOTIC THERAPIES INC  ;TRECO DOUGLAS A (US); HEARTLEIN MICHA) 23  November 1995  cited in the application  see page 108, line 2 - line 8  see page 111, line 28 - page 112, line 7  see page 114, line 18</p> <p style="text-align: center;">-----</p>	7

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 97/14866

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/14866

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 8-10 are directed to a diagnostic method practised on the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

Remark :

Although claims 13 and 14 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/14866

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9531560 A	23-11-95	US 5641670 A	24-06-97
		AU 2550495 A	05-12-95
		CA 2190289 A	23-11-95
		CN 1119545 A	03-04-96
		EP 0759082 A	26-02-97
		FI 964536 A	09-01-97
		NO 964802 A	09-01-97
		ZA 9503879 A	18-01-96
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